41-Symp
Minimizing Noise, Maximizing Information Transmission
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PLATFORM A: Membrane Protein Structure I

42-Plat
Conformational Switching of the Diphtheria Toxin T-Domain
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The diphtheria toxin T-domain translocates the catalytic C-domain across the endosomal membrane in response to acidification. To elucidate the role of histidine protonation in modulating pH-dependent membrane action of the T-domain, we have used site-directed mutagenesis coupled with spectroscopic and physiological assays. Our studies revealed several patterns of membrane action caused by replacements of various histidines, implying differential role of histidine protonation in T-domain functioning. Replacement of H257 with an arginine (but not with a glutamine) resulted in dramatic unfolding of the protein at neutral pH, accompanied by a substantial loss of helical structure and greatly increased exposure of the buried residues, W206 and W281. This unfolding and spectral shift could be reversed by the interaction of the H257R mutant with model lipid membranes. Remarkably, this greatly unfolded mutant exhibited WT-like activity in channel formation, N-terminus translocation, and cytotoxicity assays. Moreover, membrane permeabilization caused by H257R mutant occurs already at pH 6, where wild type protein is inactive. In contrast, replacing all three histidines in the C-terminus domain (H322, H323, H372) with either neutral (triple-Q mutant) or charged (triple-R mutant) residues does not result in any alterations in solution fold (judged by CD and intrinsic fluorescence data) nor in insertion of the TH8-9 helical hairpin (judged by spectroscopic responses of selectively attached external dyes); nevertheless, this produces functionally impaired mutants. We conclude that protonation of H257 acts as a major component of the pH-dependent conformational switch, resulting in destabilization of the folded structure in solution and thereby promoting the initial membrane interactions necessary for translocation. Supported by NIH GM069783(-04S1).

43-Plat
Crystal Structure of the *VIBRIO Cholerae* Cytolysin Heptameric Pore
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Many pathogenic bacteria secrete pore-forming toxins (PFTs) that specifically kill host cells. These toxins may strategically target human immune cells or more generally release materials necessary to sustain colonization of the host. PFTs are secreted as water-soluble protein molecules, bind to susceptible cells, oligomerize into ring-like pre-pore structures, and insert aphipathic channels into the membrane. In sufficient numbers, these channels are capable of lysing cells. PFT channels are characterized as α or β depending on whether the transmembrane region forms an α-helical or β-barrel structure.
The human pathogen *Vibrio cholerae* secretes a β-PFT, *Vibrio cholerae* cytolysin (VCC), which targets cholesterol and sphingolipid-containing membranes. We developed a technique for assembling milligram quantities of oligomeric VCC on asolectin/cholesterol liposomes and purified the detergent-solubilized complex by size exclusion chromatography. We crystallized and solved the high-resolution structure of the 450 kDa VCC heptamer by X-ray crystallography to 2.9 Å. This structure, together with our previous water-soluble monomer structure of VCC, represent endpoints in the assembly process and provide a detailed account of the conformational changes that occur upon channel formation. This includes substantial reorganization of the channel-forming loop and accessory lectin domains. Our structures represent the first high-resolution pair of water-soluble and assembled states for a β-PFT, and provide insight into unresolved questions regarding the mechanism of channel assembly and membrane specificity.

44-Plat
Deciphering the Subunit Stoichiometry and Structural Assembly of Bacterial ABC Transporter
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The ATP-binding-cassette (ABC) transporters are protein nanomachines associated with membranes and common to all living cells. ABC transporters utilize the energy of ATP hydrolysis to transport a variety of solutes across the membrane. Wzm (the transmembrane component) and Wzt (the nucleotide binding component) proteins constitute an ABC transporter that translocates the endotoxic polysaccharides through the inner membrane of the pathogenic bacterium *Pseudomonas aeruginosa*. To gain insights into the subunit stoichiometry and structural assembly of this ABC transporter, we reconstituted the ABC transporter in CHO cell lines. We expressed Wzm and Wzt proteins fused with green fluorescent protein (GFP_2) or its variant, yellow fluorescent protein (YFP). The three-dimensional cellular localization of the expressed ABC components were constructed by stacking images of different sections of the cells utilizing a newly developed and spectrally-resolved two-photon microscope. We next used Fluorescence Resonance Energy Transfer (FRET) to obtain a quantitative understanding of the interaction between the ABC components by calculating the apparent FRET efficiencies for single pixels. When Wzm-GFP_2 and Wzm-YFP were co-expressed, our FRET analysis indicated that Wzm self-associates within the cell membrane as an oligomer. This correlates with our biochemical characterization of membrane-extracted Wzm. When Wzt-GFP_2 and Wzm-YFP were co-expressed, our FRET analysis indicated that Wzm and Wzt interact at the inner membrane surface. This correlates with our *in vitro* studies that show Wzm and Wzt interact with each other. The distribution of FRET efficiencies were compared when Wzt-GFP_2 and Wzm-YFP or Wzt-YFP and Wzm-GFP_2 were co-expressed to study the stoichiometry of Wzm and Wzt in the ABC transporter. The combination of FRET analysis and biochemical approaches will lead to a comprehensive investigation of the structural assembly and subunit composition of this ABC transporter and ultimately other transporters.

45-Plat
Structure and Interactions of the M. tuberculosis Membrane Protein Virulence Factor Rv0899
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Tuberculosis is the seventh most common cause of death globally. The complete genome sequence of *Mycobacterium tuberculosis*, its causative agent, has allowed identification of several stress response genes that contribute to pathogenicity. Among these, the membrane protein Rv0899 is a virulence factor that confers adaptation of *M. tuberculosis* to the acidic environment of the phagosome. Its gene is restricted to pathogenic mycobacteria associated with TB and other TB-related diseases and, thus, is an attractive candidate for the development of anti-TB chemotherapeutic agents. We have determined the three dimensional structure and dynamics of residues 73-326 of this 326-residue protein. In contrast to the original predictions, residues 73-326 form a globular structure, which encompasses two independently folded domains, with mixed α/β-secondary structure, connected by a flexible linker. The central B domain (residues 80-195) reveals for the first time the fold of a BON homology domain associated with bacterial osmotic shock resistance, nodulation-specificity and lipid-binding proteins. The C-terminal domain (residues 205-326) adopts the typical fold of peptidoglycan-binding domains, and also binds peptidoglycan suggesting a periplasmic localization for this part of the protein. Residues 1-73 contain a 20-residue hydrophobic sequence that forms a transmembrane anchor. The overall architecture of the protein, its ligands, and the unexpected structure of the B domain make it difficult to reconcile a porin activity with its central domain but suggest alternative modes of membrane association.
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46-Plat
Investigating VDAC Gating via Magic Angle Spinning NMR and Electrophysiological Measurements Under Extreme pH Conditions: Implications for the Voltage-Gating Mechanism
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The voltage-dependent anion-selective channel (VDAC) is an integral membrane protein that controls transportation of metabolites across the outer